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「CASE REPORT」

Bovine leukosis in a slaughtered cow

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Abstract

A slaughtered 3 years old cow was diagnosed as bovine leukosis by postmortem inspection, agar gel immunodifusion (AGID) test, and polymerase chain reaction (PCR). It showed no special clinical signs except emaciation. Grossly, we observed enlargement of mediastinal and pelvic lymph nodes, and tubercle -like mass on pleura and lung surface, and masses on right atrium and ventricle.

Key words : Bovine leukosis, Cow, Tubercle-like mass.

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Introduction

There are four types in bovine leukosis: adult, calfhood, thymic, and skin form. The adult type is known as enzootic, but the remains are sporadic[1].

Calf form is seen less than 6 months old with a general lymphadenopathy and widespread tumor metastasis. Thymic form usually occurs in the animals of 6-8 months old with thymic tumors and sometimes extension into the thorax. Skin leukosis is seen in immature animals aged 6-24 months and is not fatal . For example, superficial cutaneous tumor developed in young adults will be disappeared spontaneously in a few weeks after being observed. There is no evidence that these forms of sporadic bovine leukosis are caused by an infectious agent[1,2].

The adult form usually occurs in the animals between 5 and 8 years old and shows weight loss, anemia, decreased milk production, enlarged external and internal lymph nodes, bulging eyes, partial paralysis of the hind legs. It can be spreaded by vaginal exudates and placentas from cows, biting

of insects, blood-contaminated needles, surgical equipment, gloves used for rectal examinations [1-3].

Diagnosis can be made by serologic, hematologic, and histologic examinations. Agar gel immunodiffusion is a good screening test for identifying infected animals or herds, and PCR is a sensitive and specific assay for direct detection of bovine leukemia viruses in peripheral blood lymphocytes [3-7].

Materials and Methods

Agar gel immunodiffusion (AGID) test

Agar gel immunodiffusion test was conducted by using Bovine leukemia antibody test kit(NVRQS, KOREA) employing 0.7% Noble agar(DIFCO) in the Borate-NaCl buffer. After the heating step has been concluded, the 0.7% solution of Noble's agar (40㎖) is poured onto petri-dishes having an outside diameter of 88㎜. The agar was then allowed to harden thoroughly . A test pattern of seven moisture-free wells must be cut to the bottom of the plate. the pattern must consist of one central well and six wells in a circle around it. The central well was filled with the antigen, others were filled with 25㎕ of the control and the required serum. The test plates were kept at room temperature in a closed humid chamber, and read 48 hours latter.

Polymerase chain reaction(PCR) assay

Nested PCR (OIE manual) [8] method was performed for PCR assay. DNA was extracted from the leukocytes collected from the bovine whole blood by using Histopaque 1077 solution (Sigma) in Leu-cosep tube. The DNA was extracted using a Mag Extractor-Genome-kit (Toyobo, Japan). The leukocyte pellets isolated from the blood were resuspended in 850ul of Lysis sol. (Toyobo) + 0.5% EDTA(Toyobo) & Binding sol. and 30㎕ of PK(Toyobo)(protein kinase). Then the solution was mixed by vortexing for 1 hour, and vortexed again after adding 40㎕ of magnetic beads. The solution was removed and the pellets were washed with

Table 1. Primer design and sequence

Oligo	Sequence	Position in K02120	PCR producton
OBELV1A	5'- CTT- TGT- GTG-CCA-AGT-CTC-CCA-GAT-ACA-3'	5029	440bp
OBELV6A	5'-CCA-ACA-TAT-AGC-ACA-GTC-TGG-GAA-GGC-3'	5442	
OBELV3	5'-CTG-TAA-ATG-GCT-ATC-CTA-AGA-TCT-ACT-GGC-3'	5065	341bp
OBELV5	5'-GAC-AGA-GGG-AAC-CCA-GTC-ACT-GTT-CAA-CTG-3'	5376	

Table 2. PCR condition

PCR	Condition
1st	94℃/45sec, 60℃/60sec, 72℃/90sec, X 5 94℃/45sec, 55℃/60sec, 72℃/90sec, X 30 72℃/420sec >/= 20℃
2nd	94℃/45sec, 58℃/60sec, 72℃/90sec, X 5 94℃/45sec, 53℃/60sec, 72℃/90sec, X 30 72℃/420sec >/= 20℃

900㎕ of washing solution and mixed for 5 seconds by vortexing again. Then the solution was removed. This procedure was conducted in twice. The pellets were washed with 900㎕ of 70% EtOH (Merk) with vortexing for 5 seconds (twice). And DNA was extracted from the pellets adding 100㎕ Sterile Water (Hyclone) with vortexing for 10 min. A electrophoresis was performed for identification of DNA extraction, this experimentation was conducted by using BLV primer made in bionier (Table 1). The operating condition of PCR equip-ment was performed as Table 2.

Results and Discussions

A 3-year old cow was slaughtered on August 24, 2007 in a slaughterhouse located in Cheongwon-gun, Chungbuk province. There were no noticeable clinical signs except slightly thin body in general inspection.

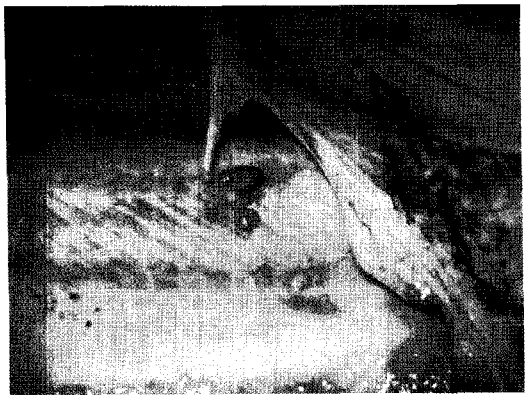

Fig 1. Tubercle like masses on pleura

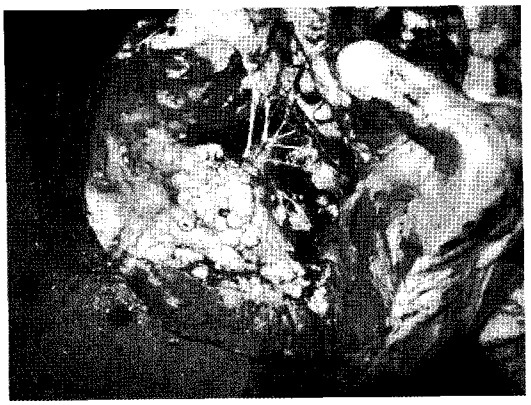

Fig 2. Masses on right atrium and ventricle

Fig 3. Enlarged lymph nodes of mediastinum and masses on lung surface

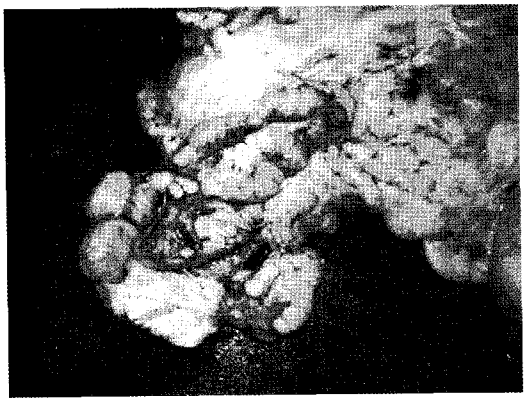

Fig 4. Masses and enlarged lymph nodes of pelvic cavity

In postmortem inspection, tubercle-like masses on pleura and lung surface (Fig 1, Fig 3),

masses on right atrium and ventricle (Fig 2), enlarged mediastinal lymph nodes (Fig 3), masses and enlarged lymph nodes on pelvic cavity (Fig 4) were observed. At first, AGID test was performed to detect antibody for BLV and confirmed seropositive reaction (Fig 5). Then nested PCR was performed with specimens from mediastinal lymph nodes. At result, 440 bp band was confirmed at 1st amplification product (Fig 6) and 341 bp band was identified at 2nd amplification product (Fig 7). Finally, the cow was diagnosed as bovine leukosis on August 27, 2007. Carcass and byproducts (about 480kg) were condemned and it was noticed to the farm.

Bovine leukosis is a chronic wasting disease with weight loss and reduction of milk production that can be responsible for major economic losses to the farmers.

Infection leads to permanent antibody response and development of persistent lymphocytosis or lymphosarcoma. Many cows remain in the preclinical stage for years (during their complete productive lifetime) without any apparent reduction in performance, but a few animals developed lymphosarcoma resulting in death within 6 months [3].

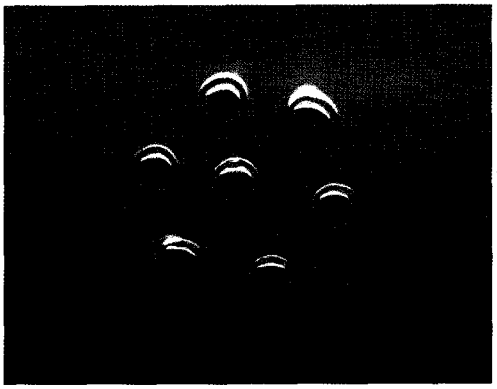

Fig 5. Rusult of AGID test

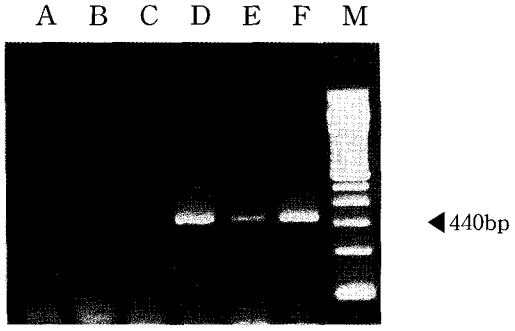

Fig 6. Result of 1st PCR

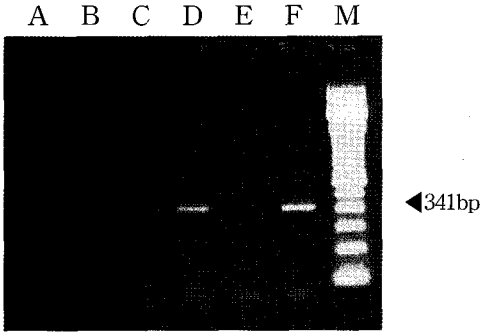

Fig 7. Result of 2nd PCR

Fig 6 & 7. Represenative patterns of agarose gel electrophoresis of PCR ampiified products to BLV provirus (A, B, C : Nagative, D, E : positive, F : positive control, M : ladder)

If infection of BLV is confirmed, the cow will be limited in moving out from the farm and depopulated for the control, but eradication is very difficult because all of the animals can not be tested in Korea.

Especially it is hard to detect a infected animal if there were not enlarged superficial lymph nodes in general inspection. Usually lymphosarcoma-like substances seem to be detected in postmortem inspection and differential diagnosis should be needed to confirm the disease.

Therefore the diagnosis of bovine leukosis in a slaughterhouse is very important to prevent the transmission of the disease to the other farmhouse.

References

1. Roger W. Blowey, A. Weaver D. 2003. *Color atlas of diseases and disorders of cattle.* 2nd ed. Mosby Co : 206-208.
2. McGavin MD, Zachary JF. 2006. *Pathologic basis of veterinary disease.* 4th ed. Mosby Co : 800
3. Merck Veterinary manual. 2006. *Bovine Leukosis.* www.merckvetmanual.com. Merck & Co. Inc.
4. Jeon MH. 1980. Bovine leukosis. *Kor J Vet Publ Hlth* 4(1): 31-39.
5. Lee HB, Choi HP, Lee KW. 1982. Outbreak of enzootic bovine leukosis in calf. *Korean J Vet Res* 22(1): 63-66.
6. Choi HY, Joung WS, Yoo KC, et al. 1992. Survey on antibodies against bovine leukemia virus in Chungbuk province. *Korean J V Serv* 15(1): 51-57.
7. Shim HS, Kook JH, Hwang YO. 1998. Seroepizootiological survey on bovine leukosis of dairy cattle in Kyunggi province. *Korean J V Serv* 21(3): 255-260
8. World organization for animal health (OIE). 2004. *Manual of diagnostic tests and vaccines for terrestrial animals.* Chapter 2.3.4 www.oie.int